METHODS OF TREATING DISEASES ASSOCIATED WITH FIBROSIS USING MODIFIED FGF-21 POLYPEPTIDES AND USES THEREOF

RELATED APPLICATION DISCLOSURE

This application is a divisional of U.S. patent application Ser. No. 15/460,917, filed Mar. 16, 2017, which is a divisional of U.S. patent application Ser. No. 15/215,329, filed Jul. 20, 2016, now U.S. Pat. No. 9,631,004, which is a continuation of U.S. patent application Ser. No. 14/921,796, filed Oct. 23, 2015, now U.S. Pat. No. 9,434,778, which claims the benefit of U.S. Provisional Appl. No. 62/141,383, filed Apr. 1, 2015, U.S. Provisional Appl. No. 62/141,337, filed Apr. 1, 2015, U.S. Provisional Appl. No. 62/068,526, filed Oct. 24, 2014, U.S. Provisional Appl. No. 62/068,523, filed Oct. 24, 2014, U.S. Provisional Appl. No. 62/068,514, filed Oct. 24, 2014, U.S. Provisional Appl. No. 62/068,296, filed Oct. 24, 2014, U.S. Provisional Appl. No. 62/068,534, filed Oct. 24, 2014, each of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING DISCLOSURE

This application includes a sequence listing which has been submitted via EFS-Web in a file named "46561o2204.txt" created May 15, 2018 and having a size of 610,931 bytes, which is hereby incorporated by reference in its entirety.

FIELD

This disclosure relates to modified FGF-21 polypeptides containing an internal deletion that is optionally replaced by a peptide and the uses thereof for treatment or prevention of diseases and disorders.

BACKGROUND

Fibroblast growth factors are polypeptides widely expressed in developing and adult tissues (Baird et al., Cancer Cells, 3:239-243, 1991) that play crucial roles in multiple physiological functions (McKeehan et al., Prog. Nucleic Acid Res. Mol. Biol. 59:135-176, 1998; Burgess, W. H. et al., Annu. Rev. Biochem. 58:575-606 (1989). According to the literature, the FGF family consists of at least twenty-two members (Reuss et al., Cell Tissue Res. 313: 139-157 (2003)).

Fibroblast growth factor 21 (FGF-21) has been described in the literature (Nishimura et al., Biochimica et Biophysica Acta, 1492:203-206 (2000); WO 01/36640; and WO 01/18172, and U.S. Patent Publication No. 20040259780, each of which is incorporated by reference herein in its entirety). Unlike other FGFs, FGF-21 has been reported not to have proliferative and tumorigenic effects (Ornitz and Itoh, Genome Biology 2001, 2(3):reviews3005.1-3005.12).

Certain FGF-21 polypeptides and uses thereof are described in U.S. Patent Publication No. 20010012628, U.S. Pat. No. 6,716,626, U.S. Patent Publication No. 2004/0259780, WO 03/011213, Kharitonenkov et al. *J Clin Invest.* 2005 June; 115(6):1627-35, WO 03/059270, U.S. Patent Publication No. 2005/0176631, WO 2005/091944, WO 2007/0293430, U.S. Patent Publication No. 2007/0293430, WO/2008/121563, U.S. Pat. No. 4,904,584, WO 99/67291, WO 99/03887, WO 00/26354, and U.S. Pat. No. 5,218,092 each of which is incorporated by reference herein in its entirety.

Human FGF-21 has been reported to have a propensity to undergo proteolysis in vivo, form aggregates in vitro, undergo deamidation (Gimeno and Moller, Trends Endocrinol Metab. 2014 June; 25(6):303-11; U.S. Pat. No. 8,361,963; Hecht et al., PLoS One. 2012; 7(11):e49345; U.S. Patent Publication No. 2007/0293430; WO 2006/0065582), potentially limiting the shelf-life of pharmaceutical compositions containing FGF-21. Aggregates and deamidated forms of therapeutic polypeptides may potentially increase immunogenicity (see U.S. Department of Health and Human Services, "Immunogenicity Assessment for Therapeutic Protein Products," August 2014; Subramanyam (ed.), "Therapeutic Protein Immunogenicity Focus Group Newsletter," American Association of Pharmaceutical Scientists, Vol. 1, Issue 3 (December 2011)).

Prior work published as WO 2008/121563 and U.S. Patent Publication No. 2008/0255045 demonstrated that certain human FGF-21 polypeptides modified to contain a non-naturally encoded amino acid linked to poly(ethylene glycol) at specified positions exhibited increased in vivo half-life and/or retained biological activity. The exemplified human FGF-21 polypeptides did not, however, include sequence deletions or substitutions described herein.

Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue. Excess deposition of fibrous tissue is associated with pathological conditions that can lead to impairment of organ or tissue function. Affected organs can include the lungs (lung or pulmonary fibrosis), liver (liver or hepatic fibrosis), kidney (kidney or renal fibrosis), and heart (cardiac fibrosis). Fibrosis can also affect other tissues and organs including joints, skin, intestine, bone marrow, and others. Exemplary fibrotic conditions or diseases include, but are not limited to, nonalcoholic steatohepatitis (NASH), which affects the liver; diabetic kidney disease and diabetic nephropathy, which affect the kidney; and metabolic heart failure, which affects the heart. For example, NASH is characterized by fat, inflammation and damage in the liver in people who consume little or no alcohol and can lead to liver cirrhosis. NASH tends to be diagnosed in overweight or obese middle-aged people who often have elevated blood lipid levels and diabetes or prediabetes.

Embodiments of the present invention address, among other things, problems associated with the activity and production of FGF-21 polypeptides, the production of an FGF-21 polypeptide with improved biological or pharmacological properties, such as improved therapeutic half-life, and methods of treating or preventing diseases and disorders.

SUMMARY

Provided herein are modified FGF-21 polypeptides comprising a polypeptide having an amino acid sequence selected from SEQ ID NOs: 1-7, except that said amino acid sequence comprises: (i) an internal deletion of between 2 and 19 amino acids (such as between 5 and 19 amino acids), wherein said internal deletion is within a region corresponding to amino acids 116 to 134 of SEQ ID NO:1, wherein said internal deletion is replaced by a replacement peptide having a length of between 0-12 amino acids; and (ii) 9 or fewer additional amino acid substitutions, deletions, and/or insertions.